

## First Report of Sudden Death Due to Myocarditis Caused by Adenovirus Serotype 3<sup>†</sup>

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**Myocarditis is a rare cause of sudden death in childhood. We describe the sudden death of a child from viral myocarditis, which we demonstrate was likely caused by an uncontrolled inflammatory response to a disseminated adenovirus serotype 3 infection originating in the tonsil.**

### CASE REPORT

An 11-year old boy collapsed suddenly at home. He was transferred to a hospital by ambulance. Cardiopulmonary resuscitation was unsuccessful, and he was pronounced dead. He had visited his general practitioner (GP) 1 day earlier with a sore throat and was treated with antibiotics for tonsillitis. No investigations were carried out by his GP or on arrival at the hospital due to his sudden death. He had no past medical history of note, and there was no family history of cardiac illness or sudden death. No recent foreign travel was reported, nor was there contact with persons from abroad. A postmortem was carried out at the direction of the coroner.

Much of the postmortem examination was normal. His tonsils were mildly enlarged and showed a surface purulent exudate. However, on postmortem examination, his heart was enlarged (weight, 214.2 g; expected weight, 124 g). On sectioning, the myocardium appeared diffusely abnormal with areas of petechiae and pallor. There was no evidence of myocardial hypertrophy. Microscopically, there was a severe myocardial infiltrate diffusely present composed of lymphocytes, histiocytes, and plasma cells with occasional eosinophils and neutrophils (Fig. 1). There were admixed areas of necrosis and hemorrhage. There was no evidence of fibrosis or myofiber hypertrophy. Immunohistochemical staining revealed that the infiltrate was predominantly lymphohistiocytic (CD45 positive). CD3 staining confirmed that this was a T-lymphocyte-mediated response (Fig. 2).

All routine investigations, including swabs for bacterial culture, blood cultures, meningococcal PCR, and pneumococcal PCR, were negative. Toxicology testing for alcohol and drugs was negative. Target-specific immunoglobulin M assays were

performed for *Mycoplasma pneumoniae*, cytomegalovirus, Epstein-Barr virus, parvovirus B19, and rubella virus, along with complement fixation testing for total antibodies to *Mycoplasma pneumoniae*, *Chlamydia* group, and adenovirus and latex agglutination testing for *Toxoplasma gondii*. All serological investigations were negative. In addition, no viruses were observed in the lung or myocardial tissue by electron microscopy. Cerebrospinal fluid Gram staining and culture were negative.

The most common viral etiologies of myocarditis are Coxsackie group B viruses and members of the adenovirus genus (3, 5). Therefore, molecular methods were employed to investigate the presence of these viruses. Total viral RNA/DNA was extracted from serum using the Qiagen QIAmp viral RNA mini kit, and total viral DNA was extracted from myocardial and pulmonary tissue using the QIAamp DNA mini kit (Qiagen) in accordance with the manufacturer's instructions. Enterovirus RNA was not detected in lung or myocardial tissue using a nested reverse transcription-PCR targeting the conserved 5' noncoding region (9). Quantitative adenovirus PCR was performed as previously described (7), using a serial dilution of cesium chloride-purified adenovirus quantitative standards (Autogen Bioclear) and hexon gene-specific primers designed to detect all 51 serotypes of adenovirus. Adenovirus DNA was not detected in pulmonary tissue; however, real-time quantitative PCR for adenovirus detected adenoviral DNA in serum (mean log value, 3.7 viral genomes per ml) and myocardial tissue (mean log value, 4.92 viral genomes per g). These viral loads are suggestive of disseminated infection rather than latent adenovirus infection.

Using the nucleic acid extract from the myocardial tissue, adenoviral typing was performed employing degenerate hexon gene-specific primers targeting a moderately conserved 475-bp region of the hexon gene used for differentiating subtypes (4). After initial identification of the group and serotype by pairwise analysis (data not shown), specific primers (5'-GATCCC GATGTTTCGCATTAT3') and 5'-TGAATGGATTACATTG TCCA3') were used to increase the amount of sequence over the hexon gene and allow a more detailed characterization.

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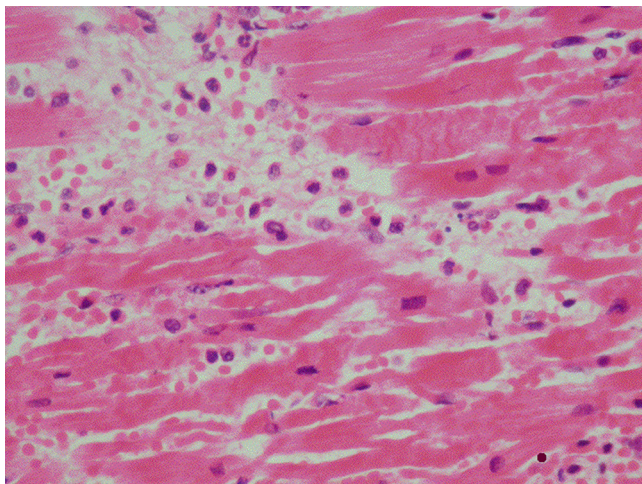


FIG. 1. Lymphohistiocytic infiltrate. Diffuse infiltrate composed of lymphocytes, histiocytes, and plasma cells with occasional eosinophils and neutrophils with admixed areas of necrosis and hemorrhage.

Nucleotide sequencing and phylogenetic analysis were subsequently performed with a 742-bp hexon sequence. The analysis indicated the strain to be 100% identical at the nucleotide level to the human adenovirus type 3 (HAdV-3) identified in respiratory disease outbreaks in southern China (20). The phylogenetic analysis (see Fig. S1 in the supplemental material) showed that the myocardial HAdV sequence is clearly genetically distinguishable (bootstrap values, >98%) from adenovirus serotype 5 and serotype 7 and segregates with other known HAdV-3 sequences. HAdV-3 is a known causative agent of acute respiratory disease, particularly in Asia; however, we believe that this is the first report to describe HAdV-3 associated with a case of fatal myocarditis in a child. A recent report from Hungary described the detection of HAdV-3 nucleic acid in explanted heart tissue of three patients with dilated cardiomyopathy (14). The GenBank accession number for the HAdV-3 identified from myocardial tissue is FJ744159. As the initial diagnosis was tonsillitis, we extracted DNA from tissue

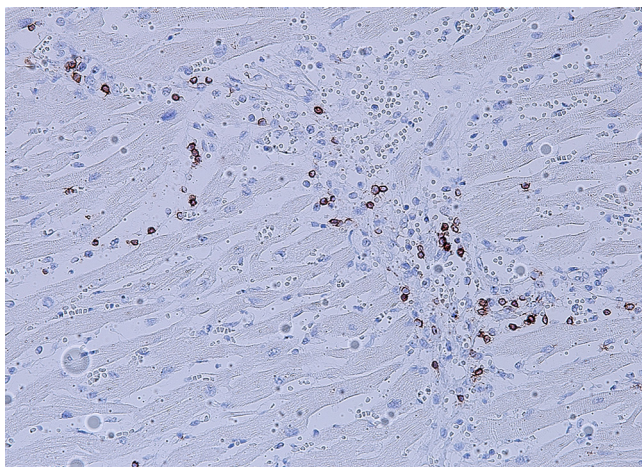


FIG. 2. Immunohistochemical staining of myocardial infiltrate. CD3 staining confirmed that it was a T-lymphocyte-mediated response.

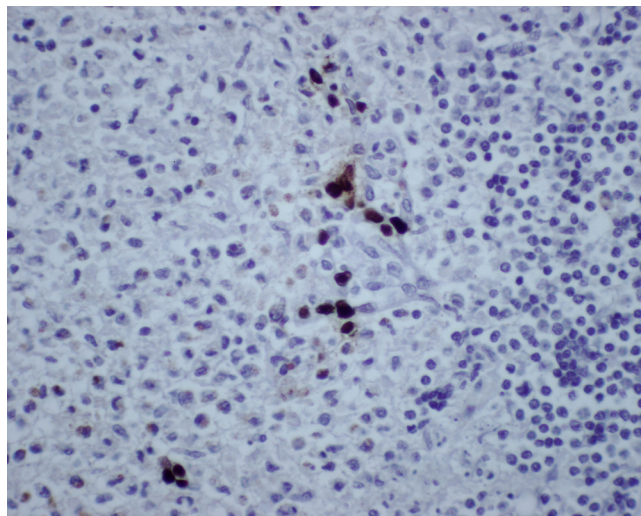


FIG. 3. Immunohistochemistry for HAdV in tonsil tissue.

derived from tonsillar material exhibiting areas of inflammation consistent with viral infection and, using the HAdV hexon gene primers described above, amplified a 401-bp hexon gene fragment 100% identical to the myocardial HAdV-3 sequence. The GenBank accession number for the tonsillar HAdV-3 hexon gene sequence is GU123900.

For immunohistochemistry, 4- $\mu$ m sections were cut from paraffin-embedded tissue blocks (tonsillar, gastric mucosal, bronchial, myocardial) for immunohistochemical staining. Sections were immunostained with an anti-adenovirus monoclonal antibody blend (Millipore MAB805) on an automated platform (BondMax system; Leica Microsystems). Briefly, cut sections were subjected to on-board dewaxing (Leica Microsystems AR9222) and antigen retrieval in enzyme 1 (Leica Microsystems AR9551) for 10 min prior to the application of the primary antibody (1:8,000 dilution) for 20 min at room temperature and detection using the Bond polymer Refine detection system (Leica Microsystems DS9800). The 3,3'-diaminobenzidine chromogen resulted in a brown end color. Sections were counterstained with hematoxylin, and both positive and negative controls (Millipore 5009-5) were used to validate the specificity of the immunohistochemical staining. The immunohistochemical analysis on the tonsillar section showed areas of clear adenovirus staining (Fig. 3). All other sections were negative by immunohistochemical analysis, including the myocardial sections (magnification,  $\times 5$ ), which we infer to be the result of the decreased assay sensitivity of the immunohistochemical analysis compared to that of endpoint and real-time PCR. Furthermore, these results are consistent with the original diagnosis of tonsillitis in this case.

To evaluate the patient's immune response, 11 cytokines (interleukin-1 $\alpha$  [IL-1 $\alpha$ ], IL-1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, gamma interferon [IFN- $\gamma$ ], and tumor necrosis factor alpha [TNF- $\alpha$ ]) were measured in serum using the multibead human cytokine Lincoplex kit (Linco Research). Multianalyte profiling was performed on a Luminex-100 system using the XY platform and Luminex IS 2.2 software. The results of the immunological investigations provided evidence of a strong inflammatory response by the presence of highly elevated serum IL-6 levels



(1,387 pg/ml; normal range, 3.1 to 8.6 pg/ml). These findings suggest a strong, uncontrolled inflammatory response resulting in sepsis due to HAdV-3 infection. Levels of IL-1 $\alpha$ , IL-1 $\beta$ , TNF- $\alpha$ , IFN- $\gamma$ , and IL-2 were undetectable. Surprisingly, IL-10, an anti-inflammatory cytokine, was moderately elevated (238 pg/ml; normal range, 0 to 5 pg/ml).

Myocarditis is defined histologically as inflammation of the myocardium. It is characterized histologically by using the Dallas classification (1) as "an inflammatory infiltrate of the myocardium with necrosis and/or degeneration of adjacent myocytes" or the WHO Marburg criterion (8) as " $\geq 14$  infiltrating leukocytes/mm<sup>2</sup>." The myocardial infiltrate may be focal or diffuse and may be composed of both acute and chronic inflammatory cells. Myocarditis may present clinically with a range of symptoms and is increasingly implicated as a cause of sudden death in infancy and childhood. A recent study of autopsies in a United Kingdom hospital reported myocarditis as the cause of death in approximately 2% of the deaths of children <18 years old, 57% of which were sudden deaths (16, 17).

A wide variety of etiological agents may cause myocarditis, ranging from systemic conditions such as sarcoidosis and Crohn's disease, therapeutic drugs, toxins, and animal/insect bites and stings, as well as multiple infectious agents. Identification of the etiological agent can be difficult due to the patchy nature of the inflammatory infiltrate and difficulty in pathogen culture/isolation. One study found that in 50% of myocarditis cases an etiological agent could not be identified although the infection was thought to be virally mediated (10). The majority of cases of myocarditis are caused by viral infection, with enteroviruses and adenoviruses accounting for most of the viral myocarditis cases investigated (2, 3, 5, 12). However, human herpesvirus 6 and parvovirus B19 are also being increasingly implicated in myocarditis (6, 11, 19).

Recently, in Cuba, an outbreak of febrile syndrome followed by acute cardiac decompensation in infants and young children has been described (13, 15). In six of the eight fatal cases, adenoviral DNA was detected in the lungs and myocardium. The etiological agent of the acute myocarditis in the fatal cases was adenovirus serotype 5 (a member of adenovirus subgenus C) in the fatal cases and adenovirus serotype 1 in the nonfatal cases (also a group C adenovirus). The outbreak in Havana was potentially attributable to the disruption of sanitary conditions by Hurricane Dennis in the preceding months and an unusually high level of adenovirus in circulation in the Cuban pediatric population (13). In contrast to the fatal cases of acute viral myocarditis described in Cuba, the virus in our case was typed as a group B adenovirus member, specifically, HAdV-3 grouped with HAdV-7, -16, -21, and -50 in subgenus B1. The 51 known serotypes of adenoviruses are responsible for a wide range of infections, and although there is no distinct correlation between serotype and disease, reports have suggested that subgenera B1 (including HAdV-3 and -7) and C are associated primarily with respiratory infection in infants and young children (2, 18). Our case was not associated with any other cases of death due to myocarditis or outbreak of illness within the immediate family or community. His immediate family mem-

bers were not screened for adenovirus, as there was no clinical indication.

Isolation of viruses from myocardial tissue at postmortem is problematic; however, the advent of highly sensitive and specific molecular techniques has important diagnostic implications. The pathogenesis of myocarditis relates to the direct effect of the infectious agent on the myocardium along with the immune and cytokine responses to the infectious agent. Sudden death may be due to a cardiac arrhythmia secondary to infiltration of the conduction system of the heart by inflammation or due to myocyte damage caused by the inflammatory infiltrate, leading to cardiac failure and cardiogenic shock. This sudden death of a young child from HAdV-3 infection of the myocardium highlights the possibility of childhood vaccination for adenoviruses to reduce these events as the number of serotypes of this viral group implicated in fatal myocarditis and other cardiomyopathies continues to grow.

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